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(54) SYSTEM AND METHOD FOR IMPROVING SAMPLE CONCENTRATION IN CAPILLARY ELECTROPHORESIS

VORRICHTUNG UND VERFAHREN ZUR VERBESSERTEN PROBENKONZENTRIERUNG BEI KAPILLARELEKTROPHORESE

AMELIORATION DES CONCENTRATIONS D'ECHANTILLONS TRAITES PAR ELECTROPHORESE CAPILLAIRE

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Descripti n

Field of th Inv ntion

The present invention relates to capillary electrophoretic systems and methods for separation and detection of sample components inside a separation column, and more particularly to improved systems and methods for increasing detectability with a sample stacking technique in capillary electrophoresis.

Background of the Invention

Capillary zone electrophoresis (CZE) is an efficient analytical separation technique which exploits the different mobilities of sample components in an electric field whereby the sample components are organized into zones in a capillary column.

A conventional CZE system widely used in practice comprises a buffer-filled capillary column with inlet and outlet ends disposed into two reservoirs, sample introduction means for injecting analyzed sample, on-column detector means for sensing the sample zones passing the detector, and high voltage means to apply a voltage to the capillary column causing the migration and separation of the sample components inside the column (see Jorgenson, J.W; and Lukacs, K.D., Science, 1983, v222, p. 266-272).

Using CZE for the analysis of very small sample volume creates a significant detection problem associated with the detection limit of conventional detectors. Along with the traditional technique of increasing the detection sensitivity of analyzed sample components through improvement of the detection systems, a different approach has been developed to increase sensitivity by concentrating the sample components into narrower zones in the capillary column. (Mikkers, F. E. P., Everaert, F.M.; Verheggen, Th P.E.M., J. Chromatography 1979, 169, 11). This on-column concentration technique sometimes called sample stacking, is obtained by applying a high voltage across a separation column which is filled with a plug of sample in a diluted buffer and surrounding which is a buffer having much higher conductivity than the sample plug.

A number of techniques using on-column concentration of sample ions at the boundary between a plug of sample in diluted buffer and the adjacent support buffer give the enhancement in detectability of the sample components. (Moring, S.E.; Colburn, T.C.; Grossman, P.D.; and Lauer, H.H.; LC-GC, 1990, 8,34; Aebersold, R. and Morrison, H.D.; J. Chromatography 1990, 516,79; Nielen, M.W.T.; J. Chromatography 1991, 542, 173).

Disadvantage of the Prior Art

In the conventional CZE technique with on-column sample concentration, the attempts to significantly increase an injection sample volume lead to breakdown in resolution. Nielen injected sample volume up to 15nl to obtain improvement of the concentration sensitivity. Better results in injecting up to 2% of the total capillary column volume were obtained by Moring, et al.

The limit in increasing the detectability of the above-described methods is the peak broadening mechanism caused by generation of laminar flow inside the capillary column. (Burgi, D.S.; and Chien, R.L.; Anal. Chem. 1991, 63, 2042). This laminar flow is generated from the mismatch of the local electro-osmotic mobilities and electric field strength between the sample buffer and the support buffer. The larger the sample volume introduced into the column, the broader the sample peaks will be. In general, a 10-fold increase in the amount of sample injected is obtainable before there is a loss in resolution due to laminar broadening. Another obstacle to a successful separation process of the sample ions is a very low strength of the electric field in the buffer bordering the long sample solution plug, and the much higher conductivity of the buffer compared to the sample plug. The electric field is low in the surrounding buffer because almost all of the electric field is dropped across the long sample plug, and that causes the electrophoretic velocity to decrease. This further limits the volume of sample solution injected into the column.

In addition, the systems for performing above methods do not provide the detection of the sample volume which allows rapid loading of the sample being introduced into the capillary column, that is very important for getting reproducability of this technique.

The article by Henk H. Lauer et al. "Analytical Aspects of an Automated Capillary Electrophoresis System" LC-GC, Vol. 8, No. 1 (1990) 34-46 describes a capillary electrophoresis system comprising a separation column having inlet and outlet ends; a support buffer within said separation column; a sample introduction means for loading a plug of a sample solution into said separation column, said sample solution being a sample diluted in a sample buffer; an injection detector means for detecting the volume of said sample diluted in said sample buffer being introduced in said separation column at said inlet end; a separation detector means for detecting the sample components at the outlet end of said separation column; and a power supply means having electrode means to apply an electric field along said separation column and switching means for switching the polarity of said electric field of said electrode means.

According to one aspect of the invention measuring means are provided for measuring the magnitude of electric current flow through said support buffer and said sample diluted in said sample buffer within said separation column. The measuring means is pr ferably connected between said power supply means in any one of the electrode means. The injection detector is preferably placed at a selected distance from the inlet end of the separation column for detecting the volume of the injected sample solution. The separation tube is preferably a

capillary tube.

According to another aspect of the invention there is provided a method for improving sample concentration in capillary electrophoresis as set out in claim 3. This method may be applied to increasing the concentration of positive or negative charged ions of the sample by concentrating ions of the appropriate polarity at the boundary between the first buffer and the sample solution. In this process, the length of the plug of the sample solution is preferably greater than 2% of a length of said separation column. The first and third voltages preferably have comparable values. The capillary tube preferably has silica walls and the second buffer preferably has a modifier to charge the silica walls of the capillary tube to the polarity of the ions being concentrated. The modifier is preferably cetyltrimethylammonium bromide.

An example of the invention will now be described with reference to the accompanying drawings in which:

Brief Description of the Drawings

FIG. 1 is a system for high performance capillary electrophoresis according to the present invention.

FIG. 2 is a schematic diagram of an injection into the capillary column of a sample diluted in sample buffer (H_2O).

FIG. 3 is a schematic diagram for extracting the sample buffer (H_2O) out of the capillary column.

FIG. 4 is a plot showing the comparison of electropherograms with and without the sample buffer extraction in a five minute injection.

FIG. 5 is a plot showing the comparison of electropherograms with and without the sample buffer extraction for the length of sample plug is on the order of 35 cm.

FIG. 6 is the electropherogram with the sample plug loading the entire separation column.

FIG. 7 is the electropherogram of positive charged ions separation.

FIG. 8 is a plot showing the comparison of a different duration for gravity injection with the sample buffer extraction.

Preferred Embodiment of the Invention

An improved method of sample concentration in CZE was performed using a CZE system of the present invention and illustrated in FIG. 1. Referring to FIG. 1 a CZE system consists of a capillary column 11 with an inlet end 12 and an outlet end 13; the inlet end 12 and the outlet end 13 are dipped in the buffer reservoirs 14 and 15 containing support buffer; sample introduction means 16 for introducing sample solution inside the capillary column 11 through the inlet end 12; a power supply means 17 having electrode means 18 and 19 which is disposed in the reservoirs 14 and 15 respectively; an injection detector means 20 for detecting the volume of sample solution being introduced by sample introduction means 16 through inlet end 12 into the capillary column 11; separation detector means 21 for detecting the sample components at the outlet end 13 of the capillary column 11; ammeter 22 connected between power supply means 17 and either electrode means 18 or 19 for monitoring current flow through electrolytes within capillary column 11.

The support buffer is supplied from the reservoir 14 hydrodynamically to the capillary column 11 which is a 100 cm long, 50µm ID, 365 µm OD fused capillary with a detector window at 35 cm from one end of the column (PolyMirco Technologies, Phoenix, AZ). Either end of the capillary column 11 might be used as the injection (inlet) side giving a detector window at 35 cm or 65 cm from the inlet end. The detector window was formed by burning off a 1 mm section of the outer polyamide coating.

Reservoir 15 at the outlet end 13 of the capillary column 11 collects the support buffer after filling the column. A high voltage is applied by power supply means 17 between the inlet end 12 and outlet end 13 of the capillary column 11 through electrode means 18 and 19, which are platinum wires preferably, for measuring the magnitude of electric current flow through the support buffer by ammeter 22.

Sample introduction means 16 is a syringe by which the sample solution is injected into the capillary column 11 until the injection detector means 20 responds to the sample solution passing through the window of the capillary column 11. This method allows 1/3 or 2/3 of the column to be filled rapidly depending on the location of the detector window to the inlet end of the capillary column. One can place the injection detection at the outlet end of the capillary column and fill the column completely up.

The alternative method of sample solution introduction is to position the sample solution vial 15 cm above the floor of the buffer reservoir 14. The inlet end 12 of the capillary column 11 is inserted into the vial and held there for 10 sec. to 20 min., which corresponds to plug lengths of 1 mm and 12cm respectively. After injection, the inlet end 12 of the capillary column 11 is returned to the buffer reservoir 14. The volume of sample solution being introduced is detected by injection detector means 20 which is an UV absorbance detector, for example, a Varian 2550 (Walnut Creek, CA) with a 100 µm slit in a modified microcell holder. The wavelength for analysis is 265 nm.

While sample stacking is being performed, the sample buffer is pushed out into the buffer reservoir 14 by applying reversed polarity high voltage by the power supply means 17, between the inlet 12 and outlet ends 13 of the capillary column 11 through electrode means 18 and 19. The current flow level through sample solution and support buffer is monitored by ammeter 22 until its magnitude reaches within 1% of th current flow valu of th support buffer.

For separation of the sample into its components, the polarity of the electrode means 18 and 19 is switched

to normal, and high voltage is applied by power supply means 17 to the capillary column 11. The detection of the sample components at the outlet end 13 of the capillary 11 is provided by separation detector means 21 which is the same type of UV absorbance detection as the injection detector means.

Sample Concentration Procedures

The essence of keeping the high sample concentrated in sharp bands constitutes the method of extracting the sample buffer from the capillary column using electro-osmotic flow after the sample is stacked into the support buffer.

With a negatively charged silica capillary wall, the negative sample ions are stacked at the end of the plug of sample buffer and will follow the plug as it moves through the column under the applied electric field. For separation of positive sample ions, the charge on the silica capillary wall is made positive by adding Tetradecyltrimethylammonium bromide (TTAB) to the buffer, for changing the direction of the electro-osmotic flow.

The first step of the concentration technique is injection of the sample diluted in sample buffer which is shown schematically in FIG. 2. The sample buffer in this case is a pure water. The experiments were conducted with stock solutions of $1.7x10^{-4}$ M PTH-Asp acid and $2.3x10^{-4}$ M PTH-Glu acid made up in HPLC grade distilled water (Aldrich, WI) then diluted to 3.4×10^{-5} M and 4.6×10^{-5} M respectively. The two positive species were 1.5×10^{-4} M PTH-Arg and 5.0×10^{-5} M PTH-His. The support buffer was 100 mM sodium phosphate adjusted to pH of 6.6 or 100mM MES/HIS adjusted to pH 6.1. All chemicals were purchased from Sigma (St. Louis, MO). Tryptic digest was done with 0.02 g of Cytachrome c (horse heart) and 0.001 g of trypsin in 10 ml of water. The digest was kept at 37C° for 28 hours, then diluted down to 5.0×10^{-5} M with distilled water. The sample prepared in water was loaded into the capillary column by gravity injection.

Since the electrophoretic velocity of the ions inside the water plug is much faster than the bulk electro-osmotic velocity, application of high voltage with reversed polarity immediately after loading the sample causes extraction of the water plug (only) from the capillary column as shown in the FIG. 3. The negative ions are stacked into a thin zone on the back side of the sample plug.

Removal of the water plug from the capillary column is controlled by applying a high voltage with reverse polarity across the capillary column while monitoring the magnitude of the electric current flow through the sample solution and support buffer inside th column, and comparing it with the magnitude of the current flow through support buffer inside the column before injecting the sample solution inside the column.

When th current flow reaches 95% to 99% of the current flow of homogeneous systems, th voltage is disconnected. For further separation into components of the stacked sample ions, it is necessary to switch polarity of the electrodes to the normal polarity and apply high voltage having a comparable value to the high voltage being applied to the capillary column before sample solution injection.

The improvement in the separation of the sampl obtained by the removal of the sample buffer (water) is shown in FIG. 4. The experiment was conducted under the following conditions: applied high voltage to the capillary column is on the order of 25kV, and current flow through the support buffer inside the column is on the order of 225μA for the phosphate buffer and 8μA for the MES/HIS buffer. The electropherograms of a long sample plug (10 min. injection) with the water still in the column and with the water removed are shown in electropherograms (a) and (b), respectively. The resolution of the two analytes is much lower in electropherogram (a) because of the large amount of water in the column. The number of theoretical plates in electropherogram (a) are $2.5x10^4$ and $2.3x10^4$ for PTH-Asp and PTH-Glu respectively. The sample peaks are broadened due to a laminar flow generated by the long plug of sample buffer. The separation of the sample is not well resolved because the bulk electro-osmotic flow is increased and the electric field in the buffer portion of the column is reduced by the large amount of water. In contrast, the number of theoretical plates in electropherogram (b), where most of the water has been removed, are $4.2x10^4$ and $4.5x10^4$ respectively for the separation of PTH-Asp and PTH-Glu. All of the compounds in the sample plug are baseline resolved, the peak heights are greater, and the peak widths are narrower. Thus by removing the excess water after the sample is concentrated, we can improve the resolution and detectability of the sample.

FIG. 5 is a comparison between the injection of 35 cm of sample solution without and with the sample buffer removed from the column. As seen in the upper electropherogram, the negative ions stack up against the sample plug and since all of the electric field is dropped across the sample plug, the ions cannot separate themselves into discrete sample zones. The lower electropherogram shows the whole process of sample solution removal and ion separation. During the first 3 minutes of the process the sample buffer is removed. After the current reaches 99% of the support buffer current, th electrodes are switched. As seen, the remaining sample buffer passes the separation detector first, then the concentrated sample ions are detected with high resolution.

FIG. 6 is an electropherogram of an injection in which the entire column is filled with sample solution. The first 7 minutes is the removal of the sample buffer. After the curr nt reaches 99% of the support buffer, the electrodes ar switched. The sample buffer peak appears at 13 minutes and the sample ions appear later with high resolution. In other words, the sampl ions in the entire column hav been concentrated into very sharp zones.

FIG. 7 is an electropherogram of two positive spe-

cies. The two sample ion are PTH-His and PTH-Arg respectively. The support buffer has 1 mM TTAB in it which reverses the electro-osmotic flow of the column.

A comparison of a tryptic digest of Cytachrome c at a 1 min. injection duration and a 10 min. injection duration with the water removed is shown in FIG. 8. One can see more peaks and greater peak heights using the concentrating method in the tryptic digest analysis; however, there is no one-to-one match of peaks between the two types of injection, possibly due to concentrating effects.

The experimental data demonstrate the improvement of the concentrating effect of sample stacking on the example of gravity injection. The sample buffer which causes a loss of resolution in the sample stacking technique is extracted by electro-osmotic flow from the separation column, and further separation of the concentrated sample zone proceeds under conventional electrophoretic conditions.

Claims

1. A capillary electrophoresis system comprising:

   a separation column (11) having inlet and outlet ends (12 and 13 respectively);
   a support buffer within said separation column;
   a sample introduction means (16) for loading a plug of a sample solution into said separation column, said sample solution being a sample diluted in a sample buffer;
   an injection detector means (20) for detecting the volume of said sample diluted in said sample buffer being introduced in said separation column at said inlet end;
   a separation detector means (21) for detecting the sample components at the outlet end of said separation column; and
   a power supply means (17) having electrode means to apply an electric field along said separation column and switching means for switching the polarity of said electric field of said electrode means characterised by measuring means (22) for measuring the magnitude of electric current flow through said support buffer and said sample diluted in said buffer within said separation column.

2. The system of claim 1 further characterised by a first (14) and a second buffer reservoir (15) for containing said support buffer wherein said electrode means of said power supply means are disposed to apply electric field along said separation column, said first and said second buffer reservoir are disposed at said inlet and said outlet end of said separation column respectively.

3. A method for improving sample concentration in capillary electrophoresis comprising steps of:

   substantially filling a separation column with a first buffer;

   measuring the magnitude of a current flow through said first buffer inside said separation column by applying a first voltage across said separation column for a period of time necessary for performing the measurement of said first current flow to determine the first current flow value;

   obtaining sample solution by preparing a sample in a second buffer, said second buffer having concentration lower than said first buffer;

   introducing a plug of said sample solution into said separation column adjacent to said first buffer;

   concentrating ions of said sample at the boundary between said first buffer and said sample solution, and substantially extracting said second buffer from said separation column by applying a second voltage across said separation column and concurrently monitoring the magnitude of current flow through said sample solution and first buffer within said separation column, said second voltage having an opposite polarity to said first voltage;

   applying a third voltage across said separation column for separating said sample into its components thereby providing a signal when each component is detected, said third voltage having opposite polarity to said second voltage.

4. The method of claim 3 wherein said first buffer has identical composition as said second buffer.

5. The method of claim 3 wherein said second buffer is pure water.

6. The method of claim 3 wherein said plug of said sample solution is introduced hydrodynamically into said separation column.

7. The method of claim 6 wherein said plug of said sample solution is introduced into said separation column until obtaining a respons from said injection detector.

8. The method of claim 6 wherein said plug of said sample solution is introduced into said s paration column until the length of said plug of said sample solution is comparabl with the length of said sep-

aration column.

9. The method of claim 3 wherein step of substantially extracting said second buffer from said separation column comprises:

applying said second voltage to achieve a second current flow having an opposite direction to said first current flow, maintaining said second voltage until said second current flow increases to a selected value, said selected value less than said first current flow value.

10. The method of claim 9 wherein said second current flow is substantially 99% of said first current flow.

Patentansprüche

1. Kapillarelektrophoresesystem, umfassend:

eine Trennsäule (11) mit Einlaß- und Auslaßenden (12 bzw. 13);
eine Trägerpufferlösung in der Trennsäule;
ein Probeneinführungsmittel (16) zum Einbringen eines Pfropfens einer Probenlösung in die Trennsäule, wobei die Probenlösung eine in einer Probenpufferlösung gelöste Probe ist;
ein Injektionsdetektormittel (20) zum Bestimmen des am Einlaßende in die Trennsäule eingeführten, in der Probenpufferlösung verdünnten Probenvolumens;
ein Trenndetektormittel (21) zum Nachweis der Probenkomponenten am Auslaßende der Trennsäule; und
ein Stromzuführungsmittel (17) mit Elektrodenmitteln zum Anlegen eines elektrischen Feldes an die Trennsäule und Schaltmitteln zum Umschalten der Polung des elektrischen Feldes der Elektrodenmittel, gekennzeichnet durch Meßmittel (22) zum Messen der Größe des elektrischen Stromes durch die Trägerpufferlösung und die in der Pufferlösung verdünnte Probe innerhalb der Trennsäule.

2. System nach Anspruch 1, weiter gekennzeichnet durch einen ersten (14) und einen zweiten Pufferlösungsbehälter (15) zum Aufnehmen der Trägerpufferlösung, in denen die Elektrodenmittel des Stromzuführungsmittels angeordnet sind, um das elektrische Feld entlang der Trennsäule anzulegen, wobei d r rste und zweite Pufferlösungsbehälter an dem Einlaßende bzw. dem Auslaßende der Tr nnsäule angeordnet sind.

3. Verfahren zum Verbessern der Probenkonzentration bei der Kapillarelektrophorese, umfassend die Schritte:

im wesentlichen Füllen einer Trennsäule mit einer ersten Pufferlösung;
Messen der Größe eines Stromes durch die erste Pufferlösung innerhalb der Trennsäule durch Anlegen einer ersten Spannung über die Trennsäule für einen Zeitraum, der zum Ausführen der Messung des ersten Stromes notwendig ist, um den ersten Stromwert zu bestimmen;
Erhalten einer Probenlösung durch Herstellen einer Probe in einer zweiten Pufferlösung, wobei die zweite Pufferlösung eine niedrigere Konzentration als die erste Pufferlösung hat;
Einführen eines Pfropfens der Probenlösung in die Trennsäule angrenzend an die erste Pufferlösung;
Konzentrieren der Ionen der Probe an der Grenze zwischen der ersten Pufferlösung und der Probenlösung und im wesentlichen Extrahieren der zweiten Pufferlösung aus der Trennsäule durch Anlegen einer zweiten Spannung an die Trennsäule und gleichzeitiges Überwachen der Größe des Stromes durch die Probenlösung und die erste Pufferlösung in der Trennsäule, wobei die zweite Spannung eine entgegengesetzte Polung zur ersten Spannung hat;
Anlegen einer dritten Spannung über die Trennsäule zum Trennen der Probe in ihre Komponenten, wodurch ein Signal beim Nachweis jeder Komponente vorgesehen wird, wobei die dritte Spannung eine zu der zweiten Spannung entgegengesetzte Polung hat.

4. Verfahren nach Anspruch 3, bei dem die erste Pufferlösung eine identische Zusammensetzung wie die zweite Pufferlösung hat.

5. Verfahren nach Anspruch 3, bei dem die zweite Pufferlösung reines Wasser ist.

6. Verfahren nach Anspruch 3, bei dem der Pfropfen der Probenlösung hydrodynamisch in die Trennsäule eingeführt wird.

7. Verfahren nach Anspruch 6, bei dem der Pfropfen der Probenlösung bis zum Erhalten einer Antwort von dem Injektionsdetektor in die Trennsäule eingeführt wird.

8. Verfahren nach Anspruch 6, bei dem der Pfropfen der Probenlösung in die Trennsäule eingeführt wird, bis die Länge des Pfropfens der Probenlösung mit der Länge der Trennsäule vergleichbar ist.

9. Verfahren nach Anspruch 3, bei dem der Schritt des im wesentlichen Extrahierens der zweiten Pufferlösung aus der Trennsäule umfaßt: Anlegen einer zweiten Spannung zum Erreichen eines zweiten

Stromes, der eine zum ersten Strom entgegengesetzte Richtung hat, Aufrechterhalten der zweiten Spannung bis der zweite Strom auf einen ausgewählten Wert ansteigt, wobei der ausgewählte Wert kleiner ist als der erste Stromwert.

10. Verfahren nach Anspruch 9, bei dem der zweite Strom im wesentlichen 99 % des ersten Stromes beträgt.

Revendications

1. Système d'électrophorèse capillaire comprenant:

une colonne de séparation (11) ayant des extrémités d'entrée et de sortie (12 et 13 respectivement);
un tampon de support dans ladite colonne de séparation;
un moyen (16) d'introduction d'échantillon pour charger un bouchon d'une solution échantillon dans ladite colonne de séparation, ladite solution échantillon étant un échantillon dilué dans un tampon d'échantillon;
un moyen (20) formant détecteur d'injection pour détecter le volume dudit échantillon dilué dans ledit tampon d'échantillon introduit dans ladite colonne de séparation à ladite extrémité d'entrée;
un moyen (21) formant détecteur de séparation pour détecter les composants d'échantillons à l'extrémité de sortie de ladite colonne de séparation; et
un moyen (17) formant source d'alimentation comportant un moyen formant électrode pour appliquer un champ électrique le long de ladite colonne de séparation et un moyen de commutation pour commuter la polarité dudit champ électrique dudit moyen formant électrode, caractérisé par un moyen de mesure (22) pour mesurer l'intensité du courant électrique qui circule dans ledit tampon de support et ledit échantillon dilué dans ledit tampon, à l'intérieur de ladite colonne de séparation.

2. Système selon la revendication 1, caractérisé en outre par un premier (14) et un second (15) réservoirs de tampon pour contenir ledit tampon de support dans lequel lesdits moyens formant électrodes dudit moyen formant source d'alimentation sont disposés pour appliquer un champ électrique le long de ladite colonne de séparation, lesdits premier et second réservoirs de tampon étant disposés à ladite extrémité d'entrée et à ladite extrémité de sortie de ladite colonn de séparation, respectivement.

3. Procédé pour amélior r la concentration d'échantillons dans une électrophorèse capillaire comprenant les étapes suivantes:

remplissage de manière substantielle d'une colonne de séparation avec un premier tampon;
mesure de l'intensité du courant qui circule dans ledit premier tampon à l'intérieur de ladite colonne de séparation par application d'une première tension à travers ladite colonne de séparation pendant un laps de temps nécessaire pour effectuer la mesure de l'intensité dudit premier courant qui circule, afin de déterminer la première valeur d'intensité du courant;
obtention d'une solution échantillon par préparation d'un échantillon dans un second tampon, ledit second tampon ayant une concentration inférieure audit premier tampon;
introduction d'un bouchon de ladite solution échantillon dans ladite colonne de séparation dans une position adjacente audit premier tampon;
concentration d'ions dudit échantillon à la limite entre ledit premier tampon et ladite solution échantillon, et extraction de manière substantielle dudit second tampon de ladite colonne de séparation par application d'une seconde tension à travers ladite colonne de séparation et contrôle simultané de l'intensité du courant qui circule dans ladite solution échantillon et le premier tampon à l'intérieur de ladite colonne de séparation, ladite seconde tension ayant une polarité opposée à ladite première tension;
application d'une troisième tension à travers ladite colonne de séparation pour séparer ledit échantillon dans ses composants, afin d'obtenir un signal lorsque chaque composant est détecté, ladite troisième tension ayant une polarité opposée à ladite seconde tension.

4. Procédé selon la revendication 3, dans lequel ledit premier tampon a une composition identique à celle dudit second tampon.

5. Procédé selon la revendication 3, dans lequel ledit second tampon est de l'eau pure.

6. Procédé selon la revendication 3, dans lequel ledit bouchon de ladite solution échantillon est introduit de manière hydrodynamique dans ladite colonne de séparation.

7. Procédé selon la revendication 6, dans lequel ledit bouchon de ladite solution échantillon est introduit dans ladite colonne d séparation jusqu'à l'obtention d'une réponse en provenance dudit détecteur d'injection.

8. Procédé selon la revendication 6, dans lequel ledit

bouchon de ladite solution échantillon est introduit dans ladite colonne de séparation jusqu'à ce que la longueur dudit bouchon de ladite solution échantillon soit comparable à la longueur de ladite colonne de séparation.

9. Procédé selon la revendication 3, dans lequel l'étape d'extraction de manière sensible dudit second tampon de ladite colonne de séparation comprend:

l'application de ladite seconde tension pour obtenir un seconde intensité de courant de circulation ayant un sens opposé à ladite première intensité du courant de circulation, le maintien de ladite seconde tension jusqu'à ce que la seconde intensité du courant de circulation croisse à une valeur sélectionnée, ladite valeur sélectionnée étant inférieure à ladite première valeur d'intensité du courant de circulation.

10. Procédé selon la revendication 9, dans lequel ladite seconde intensité du courant de circulation est sensiblement 99 % de ladite première intensité du courant de circulation.

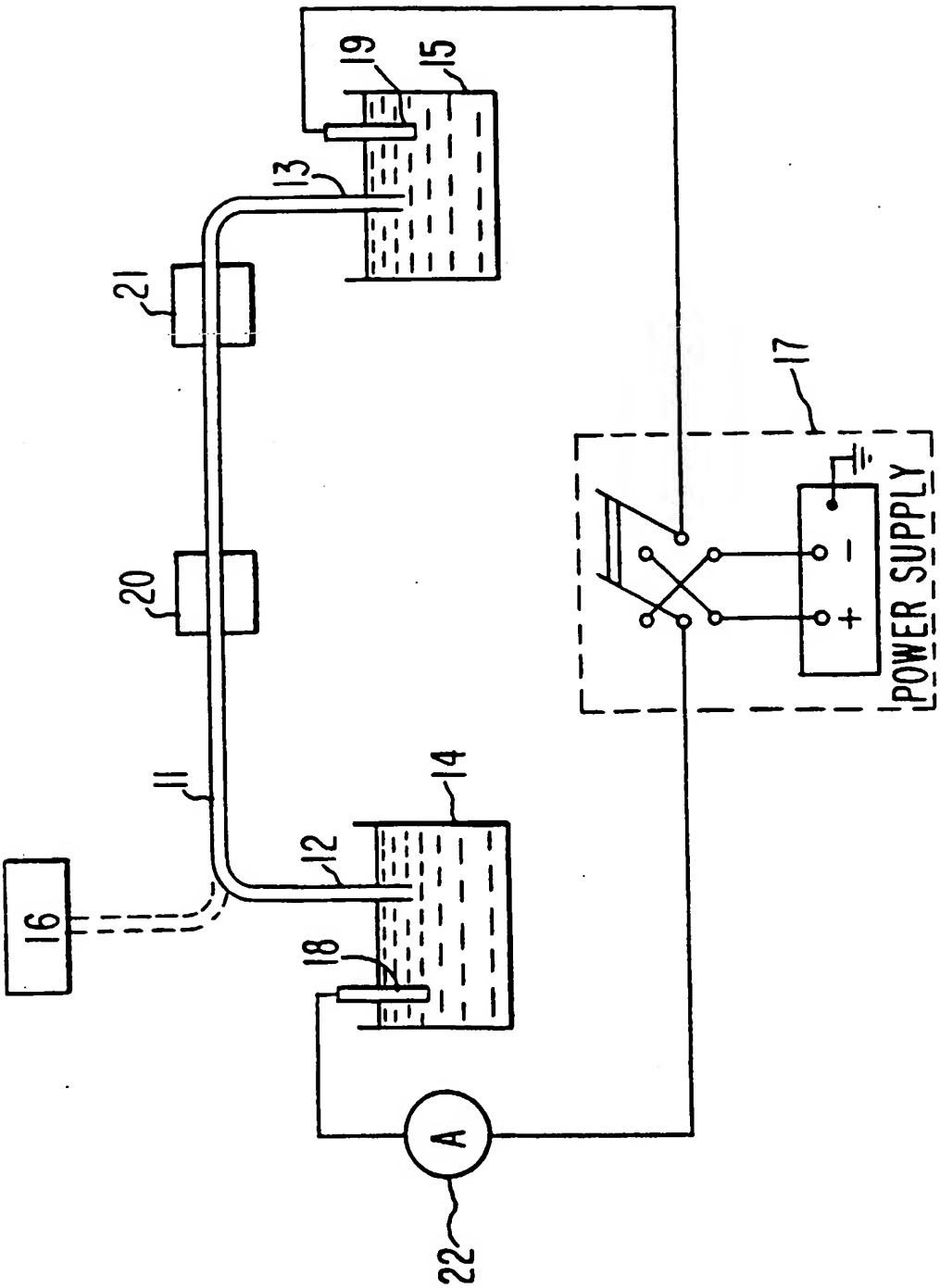

FIG.1

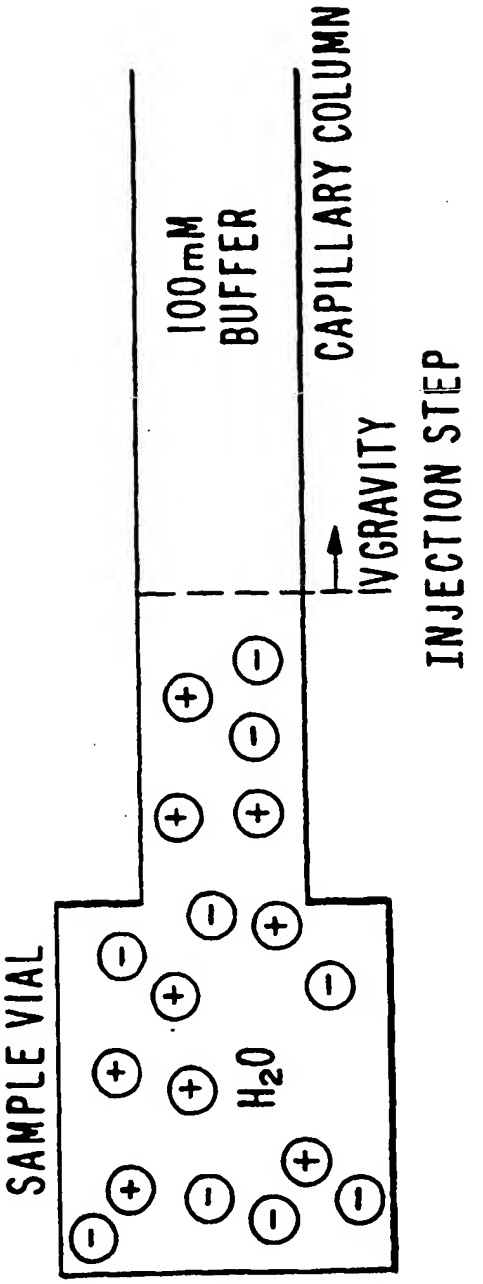
SAMPLE VIAL
100mM BUFFER
CAPILLARY COLUMN
H_2O
GRAVITY
INJECTION STEP

FIG.2

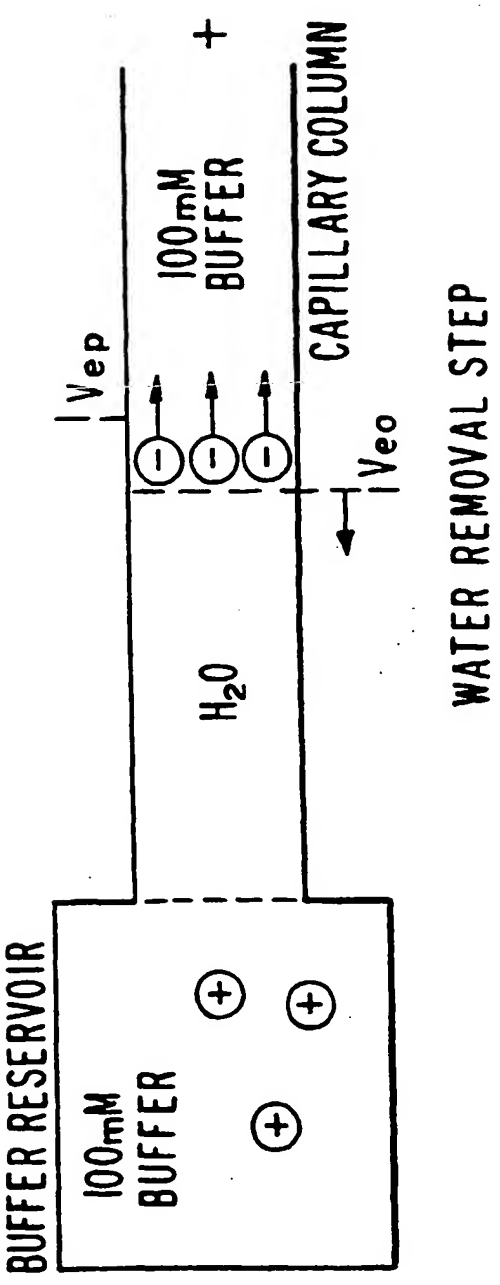
BUFFER RESERVOIR
100mM BUFFER
100mM BUFFER
CAPILLARY COLUMN
+
V_{ep}
V_{eo}
H_2O
WATER REMOVAL STEP

FIG.3

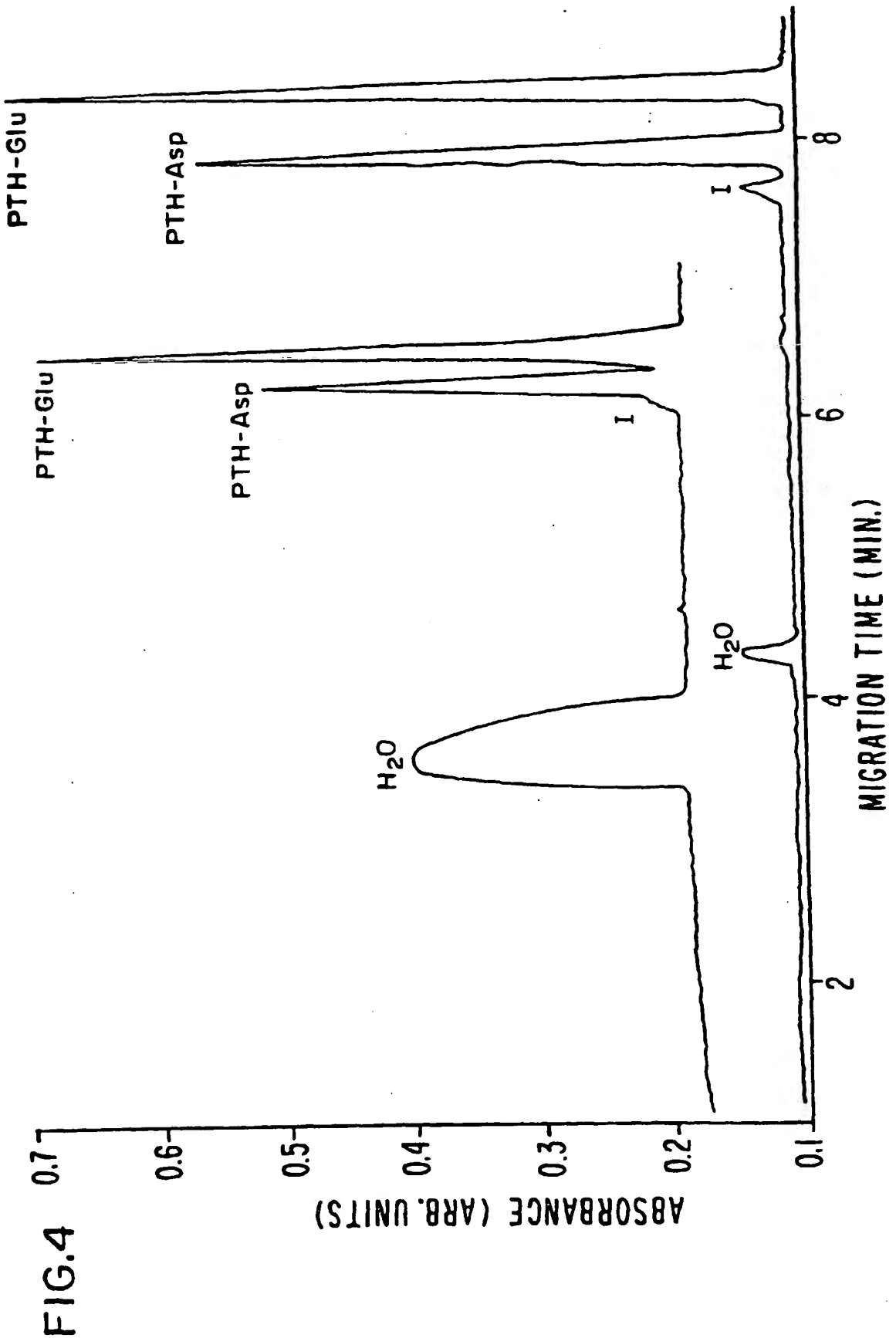
PTH-Glu
PTH-Asp
I
PTH-Glu
PTH-Asp
I
H2O
H2O
0.7
0.6
0.5
0.4
0.3
0.2
0.1
2
4
6
8
ABSORBANCE (ARB. UNITS)
MIGRATION TIME (MIN.)

FIG.4

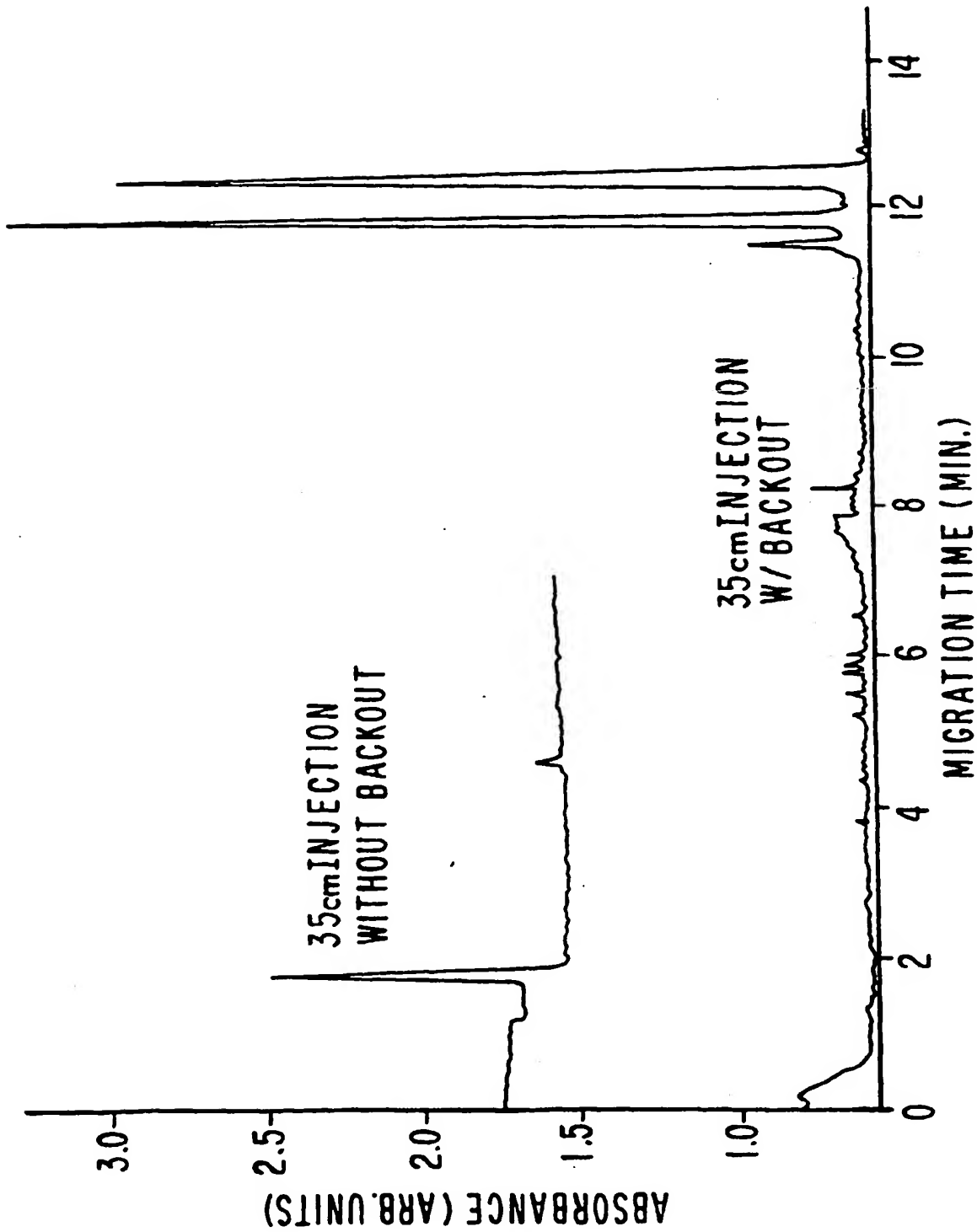
35cmINJECTION
WITHOUT BACKOUT
35cmINJECTION
W/ BACKOUT
ABSORBANCE (ARB.UNITS)
MIGRATION TIME (MIN.)
3.0
2.5
2.0
1.5
1.0
0
2
4
6
8
10
12
14

FIG.5

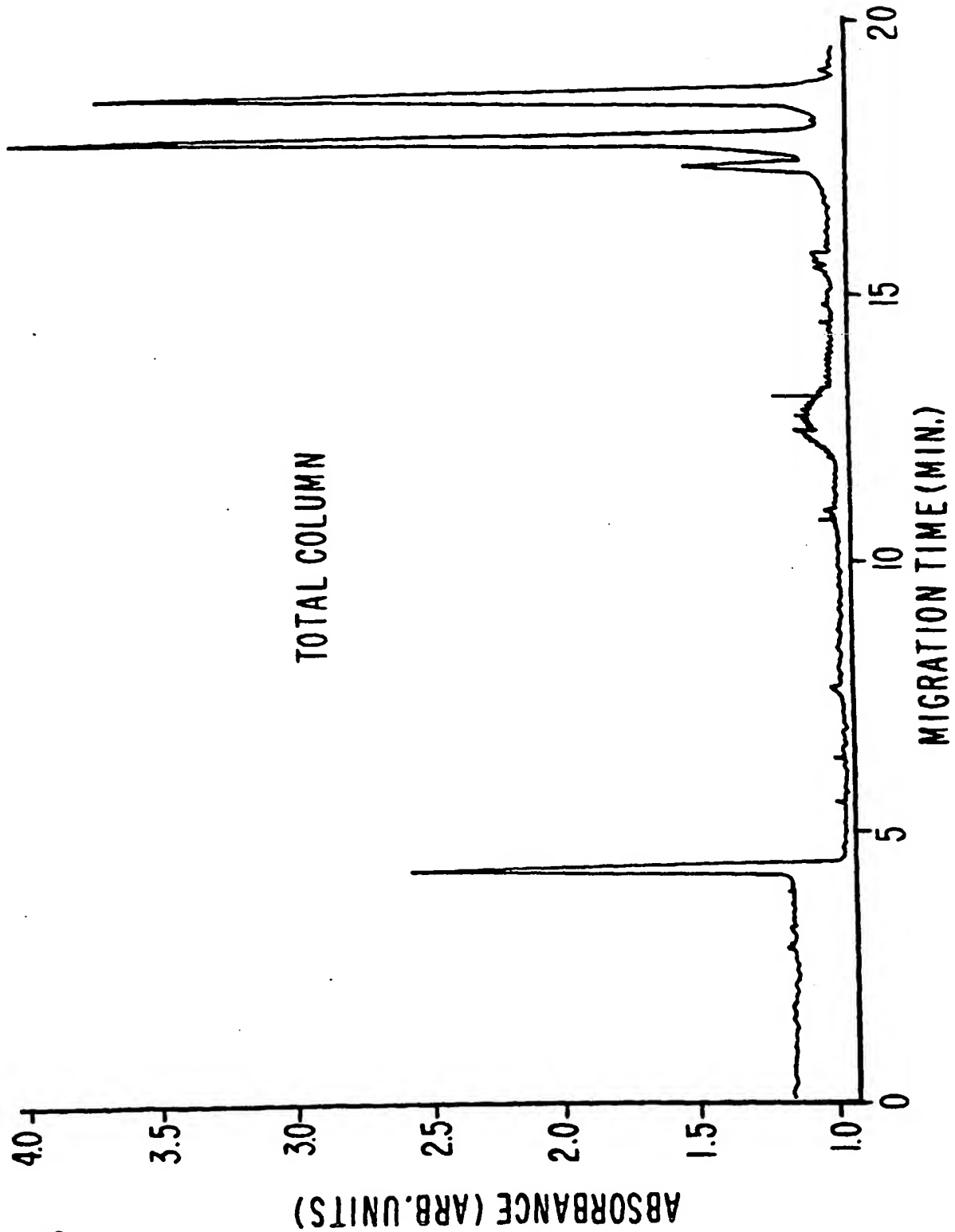
TOTAL COLUMN
ABSORBANCE (ARB.UNITS)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
5
10
15
20
MIGRATION TIME(MIN.)

FIG.6

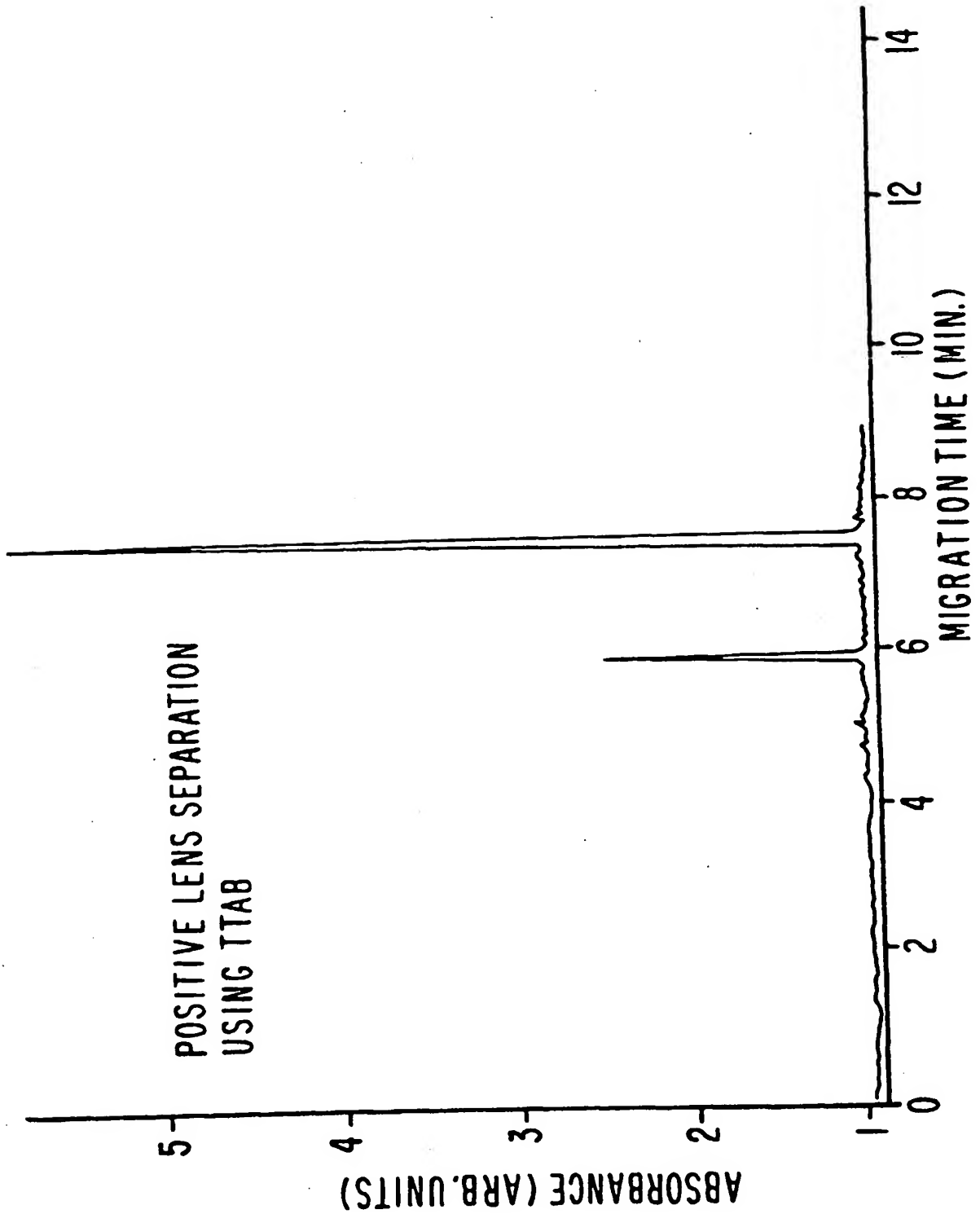
POSITIVE LENS SEPARATION
USING TTAB
ABSORBANCE (ARB. UNITS)
MIGRATION TIME (MIN.)
0
2
4
6
8
10
12
14
1
2
3
4
5

FIG. 7

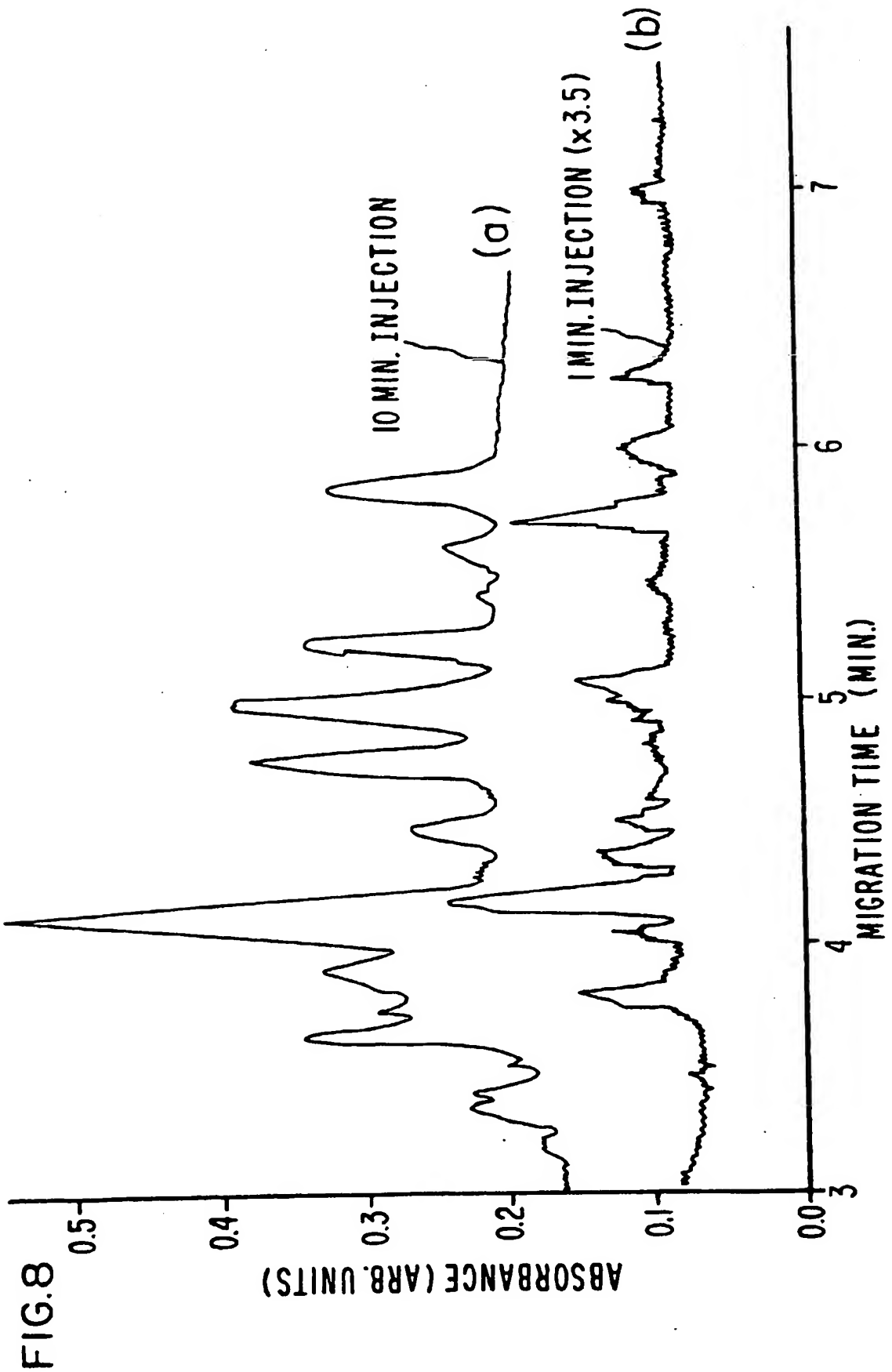
10 MIN. INJECTION
(a)
1 MIN. INJECTION (x3.5)
(b)
ABSORBANCE (ARB. UNITS)
0.0
0.1
0.2
0.3
0.4
0.5
3
4
5
6
7
MIGRATION TIME (MIN.)

FIG.8